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TITLE: ANALYTICAL AND CHARACTERIZATION STUDIES OF ORGANIC CHEMICALS, DRUGS, AND DRUG FORMULATIONS FOR WALTER REED ARMY INSTITUTE OF RESEARCH, SILVER SPRING, MD

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14. ABSTRACT
During the period October 22, 2008 to October 21, 2009, the project personnel continued to perform chemical/physical analyses on bulk pharmaceutical substances and formulated drug products, and to manufacture dosage formulations of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, etc. Specific objectives were to design, develop, validate, and apply methods to determine chemical and physical characteristics of the bulk drugs, drug products, to determine their stability under defined conditions, and to seek/work with subcontractors to produce an intravenous dosage form of artesunate.

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FOREWORD

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_____For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal law 45 CFR 46.

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_____In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI – Signature **November 2009**

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INTRODUCTION

This annual report for Contract W81XWH-09-C-0022 covers the period from October 22, 2008 — October 21, 2009. The report consists of an overview of the major activities, a listing of the specific tasks performed and reports submitted, and description of special projects performed. The report also includes a listing of personnel receiving pay from this effort and a bibliography of all publications and meeting abstracts that resulted from this contract during the report period.

This contract is concerned with analytical, characterization, and stability studies of chemicals, drugs, and drug formulations, and with development and manufacture of dosage formulations. The studies are monitored by Mr. William Y. Ellis, the Contracting Officer Representative (COR), Chief, Department of Chemical Information, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR).

The overall objective of this project is the operation of an analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products, and to develop and manufacture, in limited quantities, dosage formulations of interest of the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, anti-viral studies, etc. Specific objectives are to design, develop, validate, and execute methods to determine the following characteristics of candidate bulk pharmaceutical substances and formulated drugs, and to develop and manufacture, in limited quantities, dosage formulations.

- Identity, purity, and strength;
- Stability;
- Other physical and chemical characteristics, including weight variation, content uniformity, and other such compendial requirements;
- Qualitative and quantitative determination of impurities;
- Develop and manufacture, in limited quantities, dosage formulations; and
- Special projects not covered by the above headings.

ANNUAL REPORT (2008-2009)

OVERVIEW

During the contract period October 22, 2008 to October 21, 2009, the emphasis of our project work continues to center on the need to maintain the acceptability of the SRI manufactured artesunic acid (AS) IV drug product for its ongoing clinical trials and to select and work with a subcontractor to manufacture a second lot of the same artesunic acid IV drug product. During the 2008-2009 contract period, stability results from drug units stored through 36 months at +5° C continued to indicate chemical and biological stabilities, thereby, permitting the ongoing clinical trials to continue. Also, unformulated bulk samples of artesunic acid, treated with ethylene oxide and untreated, continue to show laboratory shelf-life stability for at least 54 months. Subcontractors have been selected to sterilize the artesunic acid, to dry-fill the unit dosage form, to prepare and fill the phosphate dissolution medium, and to perform stability studies; all phases of work are in progress.

The identification and assay of bulk drug substances and dosage formulations continued throughout the contract period.

SPECIFIC TASKS PERFORMED AND REPORTS SUBMITTED

During the contract period October 22, 2008 to October 21, 2009, the following tasks were performed and the reports submitted to the COR.

1. WR02975, Lot #401DA, primaquine phosphate tablets, Report No. 1221.

2. WR256283;BR294878, SRI Batch #14462-16, chemical stability of an artesunic acid clinical dosage form stored 28 months at +5° C, Report No. 1222. Chemical stability for the same material stored 32 months at +5° C, Report No. 1230.

3. WR256283;BQ36281, BQ37377, BQ3864, chemical stability of three lots of bulk artesunic acid stored on a laboratory shelf for 54 months, Report No. 1223.

4. WR256283;BQ37377, chemical stability of an ethylene-oxide treated bulk artesunic acid stored on a laboratory shelf for 54 months, Report No. 1224.

5. WR256283;Batch AIC 8001R, an artesunate injection formulation manufactured by IPCA Laboratories Limited of Mumbai, India, Report No. 1225.

6. WR270295;BU22894, Characterization of pamaquine monohydrochloride, Report No. 1227.

7. WR279396;BU24441, a TEVA paromomycin/gentamicin 15%/0.5% cream, TEVA lot #2314-180B, Report No. 1229.

8. WR308336;BS88570, a TEVA 15% paromomycin cream, TEVA lot #2314-113, Report No. 1231.

SPECIAL PROJECTS

During this report period, we continue to refine the sample preparation procedure of USP <788>, Particulate Matter in Injections, to accommodate characteristics of the drug units being tested. The procedure cited in USP <788> is unsuitable for our drug product because the dissolution medium supplied in our product, sodium phosphate, is a surfactant which when agitated forms air bubbles that, if not dissipated, are counted as particulates and invariably cause failure of test.

The dissolution of AS in phosphate requires agitation of the mixture to enhance liquid/solid contact because the AS powder does not easily wet. Soon after dissolution, the dissolved AS undergoes hydrolysis producing dihydroartemisinin (DHA), which has limited solubility in aqueous media, including phosphate. Because of this hydrolytic problem, a shorter dissolution time would result in a smaller amount DHA formed. To shorten the dissolution time, greater agitation of the mixture is needed. The greater the agitation, the larger number of air bubbles will form. The larger the amount of air bubbles is formed, a longer dissipation time is required, and this extended time will cause more DHA to form. Additionally, the agitation process is manual and variable; moreover, the drug units being tested contain varying amounts of AS, a situation that further compounds the problem because the solid/solvent ratios differ.

As a result of many trials, we wrote an SOP for sample preparation that calls for agitation by rotating the mixtures by hand for at least 15 min or until all solutions are visibly complete. Because the USP monograph calls for the dissolution of 10 vials, at least three (3) chemists are needed to simultaneously prepare the 10 solutions. After all 10 solutions are prepared, they are gently combined and the solution left undisturbed for an hour, before aliquots are removed for particulate counting.

A great deal of effort and time were spent on selecting and working with subcontractors to manufacture a second batch of artesunate IV dosage form. Our drug product consists of two components: a powder API and a phosphate dissolution medium. Selecting the laboratory to manufacture the phosphate dissolution medium was straightforward because we had previously contracted

Afton Scientific Corporation to manufacture our 2004 lot. Selecting the laboratory to manufacture the API-fill component was more involved because (1) powder fill of an IV product is uncommon and (2) our production lot is small, only 10,000 units.

After much searching, we narrowed our choices to two firms, both of which we site-visited. Although the overall costs from these two bidders did not differ significantly, the facilities and the organization of one were clearly superior to the other. The winning subcontractor was Dalton Pharmaceutical Services in Toronto, ON, Canada.

In addition to these two subcontracts, we need a third to perform the ethylene oxide sterilization of the artesunate and of the mannitol, the surrogate for validation of the sterile filling procedure. Because we had previously contracted Steris Isomedix Services to successfully treat the same chemicals with ethylene oxide, we wanted to stay with the same laboratory for sterilization, although the quantity of each chemical is substantially larger this time around.

Even though our group was responsible for the procurement of the 2004 artesunate IV dosage form, Batch 14462-16, we were only indirectly involved with day-to-day, detailed activities, with which we now must deal. Getting all the subcontractors on the same page, obtaining all approvals/signatures, coordinating scheduling of all activities, and interacting with all subcontractors to ensure all technical and regulatory requirements are met have required much of our effort and time during the current contract year.

In addition to the continuing and new efforts on the artesunate dosage form, we continue to provide analytical chemical service to the Army. A case of interest was with a 15%/0.5% paromomycin/gentamicin cream. The manufacturer had mistakenly reported a significant amount of impurities in one of the two antibiotics. In actuality, however, the reported impurities were actually components of the second antibiotic.

PUBLICATIONS AND PRESENTATION

A publication entitled “Chromatographic separation and NMR characterization of the isomers of MMB-4, a bis-(pyridiniumaldoxime)” by Patrick Macauley, Ronald Spanggord, William Chrisman, Peter Lim, and William Ellis appeared in Journal of Pharmaceutical and Biomedical Analysis, Volume 49, issue 4, 1 May 2009, pages 889-894.

A poster entitled “Chromatographic separation and NMR characterization of the isomers of MMB-4, a bis-(pyridiniumaldoxime)” was presented by William Y. Ellis and Peter Lim at the 19th Asian Pacific Military Medicine Conference, April 2009.

AWARDS

No awards were received during the report period.

PERSONNEL

A listing of personnel who received major contract support during the report period is as follows:

Peter Lim, P.I.
Ronald Spanggord, Assistant P.I.
Patrick Macauley, Chemist
Jennifer Wang, Chemist
Katherine Irwin, Chemist

SUMMARY/CONCLUSIONS

Results from a continuing stability study on the artesunate IV dosage stored at 5° C have shown stability for at least 36 months and enabled its clinical use to continue. Results from a continuing shelf-life stability study on bulk artesunate have shown stability for at least 54 months.

Manufacture of a second lot of the 110-mg artesunate IV dosage form is on its way. Times of product release, end of December 2009 for the solvent component and mid-February 2010 for the solid artesunate component, are on schedule.

The project team continues to provide solutions to the Army's analytical chemical problems.

Respectfully Submitted:

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